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Analysis of 14 CAG Repeat-Containing Genes in Schizophrenia

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Recently, it has been suggested that trinucleotide repeat-containing genes may be involved in the etiology of schizophrenia. This study was aimed at investigating putative associations between allelic variants or expansions of CAG repeat-containing genes (CAGrCG) and schizophrenia or its variability with respect to responsiveness to conventional neuroleptics. CAG repeat allelic variants of 14 expressed sequences were compared among three groups of subjects: neuroleptic-responder (R; n = 43) and neuroleptic-nonresponder (NR; n = 63) schizophrenic patients, and a control group (C; n = 122). No CAG expansions, in the range of those observed in neurodegenerative diseases, were identified in these 14 expressed sequences. The sizes of CAG repeat for the *hGT1* gene were marginally different among the three groups of subjects (Kruskal-Wallis H (2, 456) = 10.48, Bonferroni corrected P = 0.047). Comparisons among the different groups indicated that neuroleptic responders have shorter alleles compared to controls (Mann-Whitney adjusted Z = –3.23, P = 0.0012). NR patients were not different from controls. These preliminary results suggest that the *hGT1* gene, or a gene in its vicinity, may be involved in the etiology of schizophrenia or in modifying the disease phenotype with regard to outcome and/or neuroleptic responsiveness. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 88:694–699, 1999.

KEY WORDS: anticipation; CAG repeats; *hGT1* gene; schizophrenia

INTRODUCTION

Although genetic factors are known to play a major role in the etiology of schizophrenia [Kendler and Diehl, 1993], identification of susceptibility genes has been difficult [Karayiorgou and Gogos, 1997]. This may in part result from the variability of the schizophrenia phenotype. One possible strategy to reduce heterogeneity in schizophrenia is to classify schizophrenic patients according to their long-term outcome and response to neuroleptic medication [Brown and Herz, 1988, 1989; Brown, 1995]. Indeed, 15 to 25% of schizophrenic subjects remain severely symptomatic in spite of multiple and adequate treatment with neuroleptic medication [Brenner et al., 1990]. Compared to neuroleptic nonresponders (NR), the group of responders (R) is characterized by a later age of onset [Meltzer et al., 1997], a higher female-to-male ratio [Szymanski et al., 1995, 1996], a better long-term outcome [Breier et al., 1991], and reports of more frequently disturbed indices of dopamine neurotransmission [Mazure et al., 1991].

CAG repeat expansions were reported to be associated with genetic anticipation [Paulson and Fischbeck, 1996], a feature believed to be present in some families of schizophrenic patients [Bassett and Husted, 1997; Gorwood et al., 1996], and longer CAG repeat genomic sequences were reported to be more frequent in schizophrenic patients compared to normal controls [O'Donovan et al., 1996; Burgess et al., 1998]. In addition, using the 1C2 antibody, we [Joober et al., 1999] and others [Morinière et al., 1999] identified unrelated schizophrenic patients carrying a 50-kDa protein that contains a potentially expanded polyglutamine tract. Although promising, the results of these studies have been challenged [Huang and Vieland, 1997; Vincent et al., 1996, 1998; Sidransky et al., 1998] as they only represent indirect evidence for the involvement of

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CAGrCG in schizophrenia. If one or several CAG repeat expansions and/or allelic variants are involved in the pathogenesis of schizophrenia, it should be possible to identify them using direct genotyping in unrelated schizophrenic patients in comparison to healthy volunteers. In this study, we genotyped schizophrenic patients selected on the basis of their therapeutic response to typical neuroleptics, for CAG repeat-containing genes. Here, we report results obtained using an initial set of 14 CAG repeat-containing ESTs/genes identified through database searches.

MATERIALS AND METHODS

Subjects

NR patients were selected from a list of schizophrenic patients identified as candidates for treatment or treated with atypical neuroleptics because of treatment resistance. Three institutions provided these NR patients: Douglas Hospital, Clinique Jeunes Adultes of L.H. Lafontaine Hospital, and the Schizophrenia Clinic of the Royal Ottawa Hospital. R patients were selected from a list of all patients who were considered very good responders to neuroleptics by their treating physician and/or nurse and who were followed in the outpatient clinics attached to the Douglas and Lafontaine hospitals. The Douglas Hospital (McGill University) and Lafontaine Hospital (University of Montreal) are the only two university-affiliated psychiatric hospitals for the Montreal intra muros region. Three hundred thirty-three schizophrenic patients were identified as potential subjects for this study. One hundred twenty-three patients did not meet criteria for schizophrenia or could not be classified as neuroleptic-nonresponders or -responders (see criteria below). Eighty-five and 125 patients, respectively, met the criteria for R and NR schizophrenia. Forty-two R and 62 NR patients were not included in the study because they either declined to participate or met other exclusion criteria.

The criteria for response or resistance to conventional neuroleptics were as follows.

NR schizophrenic patients. None had experienced remission of psychotic symptoms within the past 2 years. In the preceding 5 years, all NR patients had undergone at least three periods of treatment with conventional neuroleptics from at least two distinct families of drugs at a dose equal to, or greater than, 750 mg chlorpromazine (CPZ) equivalents on monotherapy, or 1,000 mg CPZ equivalents when a combination of neuroleptics was used for a continuous period of at least 6 weeks, and which resulted in no significant decrease in symptoms. Finally, all patients were unable to function without supervision in all, or nearly all, domains of social and vocational activities and had a global assessment score (GAS) [Endicott et al. 1976] <40 within the last 12 months. Decision for nonresponse was established by the research psychiatrist upon review of medical records, with particular attention to i) unusual thought content, ii) hallucinations, iii) conceptual disorganization, iv) motor retardation, and v) emotional withdrawal (items 3, 4, 12, 13, and 15 of the Brief Psychiatric Rating Scale; BPRS) [Woerner et al., 1988]. At the time of enrollment, a minimal score of 4 (moderate to severe) on at least three of the five BPRS items (3, 4, 12, 13, 15), a total BPRS score of at least 45 and/or a CGI (clinical global impression) score of at least 5 (markedly ill) were required. The criteria for treatment resistance were derived from those of Kane et al. [1988].

R schizophrenic patients. All patients had been admitted at least once to a psychiatric institution because of an acute psychotic episode. During each hospitalization, patients experienced a full or partial remission in response to treatment with conventional neuroleptics within 6 to 8 weeks of continuous treatment. All patients were able to function autonomously with only occasional supervision in all, or nearly all, domains of social and vocational activities. None of the R patients had to be admitted to hospitals because of psychotic exacerbation while under continuous neuroleptic treatment. All R patients had at least one psychotic relapse when neuroleptic medication was reduced or discontinued. Remission was defined as a complete or quasi-complete disappearance of schizophrenic symptoms, with limited residual symptoms, based on the treating psychiatrist clinical evaluation and hospital records. At the time of enrollment, total BPRS scores were less than 30 with no more than one item scoring 4 and/or a CGI score less than 3 (borderline mentally ill).

Patients were directly interviewed using the Diagnostic Interview for Genetic Studies (DIGS) [Nurnberger et al., 1994] and their medical records were comprehensively reviewed by a research psychiatrist. Complementary information from the treating physician and nurses in charge was obtained whenever necessary. Diagnosis was based on all the available data and the severity of the clinical syndrome at the time of examination was evaluated by the BPRS.

Controls. Controls were 122 subjects screened for DSM-IV axis I mental disorders using the DIGS. All subjects, except for one responder patient, were Caucasian with a majority (52%) of French Canadians. All participating subjects gave their informed consent. Consent was obtained from both the patient and the legal guardian for incompetent patients. The project was approved by the Research Ethics Board of each participating institution.

DNA Analysis

Sequences potentially encoding polymorphic polyglutamine tracts were identified using Basic Local Alignment Search Tool (BLAST) [Altschul et al., 1990]. Sequences containing homopolymer tracts of >7 CAG or CAA repeats in a row or potentially encoding a tract of >12 glutamine residues were used to design polymerase chain reaction (PCR) primers able to amplify the CAG and/or CAA repeats. Fourteen different candidate-expressed CAG repeats were identified and analyzed (see Table I for primer pairs and PCR conditions). Of these, nine contained a polymorphic CAG repeat.

Genomic DNA was isolated from peripheral lympho-

TABLE I. CAG-Containing Genes Tested*

GB ID	G	NQ	AT	Primer sequences, homology/function	NA	% heterozygous	CL
T08930	+	15	60	5′-AGCCCCCACCACCACCTCAACAG-3′ 5′-TGGCCAAAGGGGACAAACATCTGG-3′ Homologous of *Xenopus* elv-type ribonucleoprotein ETR-1 (U16800).	1	0	—
R98242	–	27	58	5′-AGCCGCCACAGCAGCATAGAGTA-3′ 5′-TGAATGCCAGAAGGTTGTGGTTGA-3′ Probable 60S Ribosomal protein L14EA (*Saccharomyces cerevisiae*).	1	0	—
L37868	+	21	64	5′-CAGGGCGGCCGCGGAGACGA-3′ 5′-CGGGTCCCGGGTGGTGGTTAGC-3′ N-Oct-3 transcription factor.	1	0	6
U23863	+	26	61.5	5′-CCAGGCAGGCGTCCGTTCAA-3′ 5′-GTTGGGGAGGAGCCGCCTGTT-3′ Homologous to KIAA0192 gene (D83783); function unknown.	2	3.3	1
U23862	+	7	62	5′-CAGCAGCAGCTCCAGCCCTTTCA-3′ 5′-CAGCGCCTGTGACGTCTGACTCAA-3′ No known homology.	1	0	7
N55395	+	15	62	5′-ACCCGCCTGATCTCCAACAACAAG-3′ 5′-ACGGGGTCAGGTCAAAGGAACACT-3′ Contain zing finger domains, possible transcription factor.	2	23.3	12
L10379	+	20	65	5′-CGCGGCGTCAATGAGCGCAAAGTA-3′ 5′-CACGGCCGCGGAAGCAGATACCAG-3′ Partial homology to nicotinic acetylcholine receptor alpha 4 subunit.	6	31.8	16q 13
Z78314	+	17	57	5′-GGGTGTGCCCAGCTCTGANA-3′ 5′-TTAAAGGGGAGACCAATTTGAAGT-3′ Glial derived nexine precursor. Promotes neurite extension.	5	26.1	2
T90581	–	10	58	5′-GCGCTGCAGAGCAAGCACTC-3′ 5′-TGTTGCCTGAANTTTGAATGAATG-3′ No known homology.	1	0	—
L10375	–	13	62	5′-TGGGCCAAGCTCCGGAATGTTGT-3′ 5′-TGGCGCTGAAGAGGGCGAGTAGG-3′ No known homology.	4	87.5	—
X82209	+	26	65	5′-CCGGCCCCGGACCACCAGTC-3′ 5′-CTGCCGCCTTCGCGCTCAAAGTTC-3′ Human meningioma 1 gene (AC000105).	5	39.5	22
G08295	+	22	65	5′-TATTGGCCAATCCAATCTGT-3′ 5′-CAAGAATGCAATGAGCTGTG-3′ No known homology.	19	81.1	3
M34960	+	38	62	5′-CTGTCTATTTTGGAAGAGCAACAAAGG-3′ 5′-CTGCTGGGACGTTGACTGCTGAAC-3′ TFIID TATA-binding protein, general transcription factor.	14	81.7	6
G09710	+	13	61	5′-GGGGCAGCGGGTCCAGAATCTTC-3′ 5′-CTGGCCTTGCTGCCCGTAGTGCT-3′ Homolog of murine GT1 gene (D29801).	6	51.5	17

*GB ID indicates GenBank accession number; G, genomic PCR; NQ, number of encoded polyglutamine as deduced from the sequence found in the databases; AT, annealing temperature; NA, number of alleles observed in the present study; % Heterozygous, percentage of heterozygous subjects from the present study; CL, chromosome location reported in the databases.

cytes using standard methods [Sambrook et al. 1989]. CAG repeats were amplified by PCR using specific primers and annealing temperature. PCR was performed in a total volume of 13 μl containing 30 ng of human genomic DNA, 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 1% dimethylsulfoxide, 250 mM each of dCTP, dGTP, and dTTP, 25 mM dATP, 1.5 μCi alpha 35S-dATP, 100 ng of each primer, and 3 units of Taq polymerase (Perkin-Elmer). DNA was denatured at 94°C for 5 min, then subjected to 30 cycles each comprised of 1 min denaturation at 94°C, 1 min annealing at the optimized annealing temperature for each primer pair, and 1 min elongation at 72°C. This was followed by a final extension at 72°C for 5 min. PCR products were electrophoresed on denaturing 6% polyacrylamide gels and visualized by autoradiography. Absolute allele sizes were estimated according to an M13 sequence ladder. Since differences in absolute allele sizes were in all cases multiples of 3 base pairs, we assumed that variations in allele sizes were due to differences in the number of trinucleotide repeat units in the amplified sequences. The clinical assessments were performed prior to any genotyping. Individuals blind to the status of the subjects read all genotypes.

Positive controls (DNA from patients having a spinocerebellar ataxia type 3) were genotyped to ensure that our standard protocol allows the detection of expanded CAG repeats in the range observed in neurodegenerative diseases.

Statistical Analysis

By convention, we designated the most common allele as 0, with less common alleles as positive or negative integers according to their number of trinucleotide repeats (e.g., if allele 0 had 20 repeats, allele +2 and −2 would have respectively 22 and 18 repeats). We used the Kruskal-Wallis nonparametric analysis of variance to compare the size of the CAG repeats in the three groups of patients. Any finding suggestive of association was followed by pair-wise (R vs. C, NR vs. C, R vs. NR) comparisons of the CAG repeat size using the Mann-Whitney U test adjusted for ties. Although the appropriate correction for multiple testing in our experiment should take into account the potential number of expressed CAG repeats in the human genome, it is possible that such a stringent approach would prevent us from identifying association between schizophrenia and genes with minor effects. Correcting only for the number of informative genes tested in this experiment (9 of the 14 genes were successfully amplified and were polymorphic) and considering any positive finding as suggestive of association that needs to be further investigated, using independent samples, was thought to represent a reasonable tradeoff between the risk of false positives and the risk of missing a real association. Hence, we opted to set the *P*-value at ≤ 0.0055 (nine informative genes were tested) as suggestive of an association. Because our sample contained an important number of subjects with French Canadian ethnicity, we reanalyzed our results after stratifying subjects with respect to ethnicity (French Canadian vs. non-French Canadian). Besides controlling for population stratification, due to reports of a strong founder effect described in the French Canadian population, this approach is thought to potentially increase statistical power in the case of linkage disequilibrium between one of the studied genes and a gene involved in the familial transmission of schizophrenia.

RESULTS

R and NR schizophrenic patients did not differ according to age and gender distributions. Controls were significantly older and included more females. In keeping with the study design, the two groups of patients differed significantly according to the severity of psychosis, the percentage of time spent as inpatients since their first contact with the psychiatric institution, and their age at first contact with psychiatric care facilities (see Table II).

No CAG repeat expansions were detected in the 14 ESTs. The size of the CAG repeat in the EST G09710 (locus GCT10D04) showed an overall significant difference among the three groups of subjects (Kruskal-Wallis H (2, 456) = 10.48, $P = 0.0053$). Subsequent pair-wise comparisons indicated that the CAG repeat size was significantly shorter in the R patients compared to controls (Mann-Whitney adjusted Z = −3.23, $P = 0.0012$) and tended to be shorter in R compared to NR patients (adjusted Z = −2.01, $P = 0.043$). NR patients were no different from controls (Fig. 1).

TABLE II. Demographic and Clinical Characteristics*

	NR (n = 63)	R (n = 43)	C (n = 122)
Age (years)	38.9 ± 6.8	40.6 ± 10.4	43.9 ± 12.4*
Gender, % males	74.5%	67.5%	45.5%**
FC/OC	28/35	27/16	65/57
Age at 1C (years)	18.1 ± 3.9 (54)	24.2 ± 4.8*	NA
% Time as in-patient	62% (60)	8.2%*	NA
BPRS total score	48.9 ± 8.9 (52)	24.4 ± 3.9*	NA

R, Neuroleptic-responder schizophrenic patients; NR, nonresponder schizophrenic patients; C, screened controls; FC/OC, French Canadian/other Caucasians; 1C, first contact with psychiatric care; BPRS, Brief Psychiatric Rating Scale. Numbers are Mean ± SD (n) or percentages. Asterisks in the second column indicate significance level for comparison between responder vs. nonresponder patients and those in the third column are for global comparison among the three groups of subjects. *P <0.05 using ANOVA; **P <0.05 using χ^2 statistic with the appropriate degree of freedom.

To confirm the size and the sequence of the alleles, we sequenced each allele from several different subjects. All alleles had a $(CAG)_nCAA$ sequence (n = 13 for allele 0) except for allele −1 where the sequence was interrupted by a CAA trinucleotide [$CAGCAA(CAG)_{10}CAA$]. Because allele −1 accounted for most of the size difference between R and controls, we reanalyzed the data under the hypothesis that allele −1 (but not the other short alleles) is associated to neuroleptic-responsive schizophrenia or is in linkage desequilibrium with a gene involved in neuroleptic-responsive schizophrenia. Allele −1 was over represented in R schizophrenic patients compared to controls ($\chi^2 = 8.19$, df = 1, $P = 0.004$).

The same pattern of results was observed, though only at a trend level, both in terms of allele size (CAG repeats were shorter in R vs. C: Z = −2,56, $P = 0.009$) and allele frequency (allele −1 tended to be more frequent in R vs. C: $\chi^2 = 4.39$, df = 1, $P = 0.035$) when the

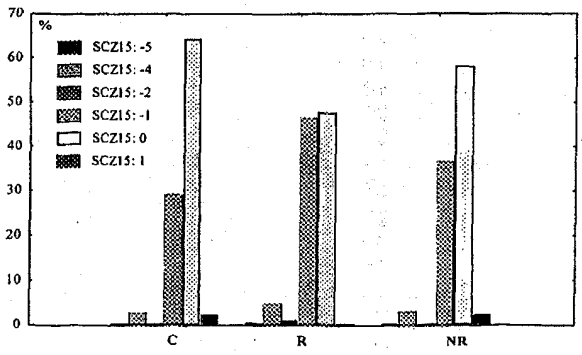

Fig. 1. CAG repeat allelic distribution (%) of the *hGT1* gene. R indicates neuroleptic-responder; NR, -non-responder schizophrenic patients; C, screened controls.

analyses were restricted to the subjects with French Canadian ethnicity.

A sequence homology search was performed using the GCT10D04 nucleic acid sequence (GenBank accession no. G09710) against the nonredundant nucleic acid database (BLASTn, GenBank). The GCT10D04 sequence was 84% identical to a mouse gene (*GT1*, GenBank D29801) from which is transcribed a 7.2-kb cDNA encoding a 196-kDa protein of unknown function, suggesting that GCT10D04 represented a portion of the human homologue, which we termed *hGT1*. This gene was mapped to chromosome 17q11.2 by Philibert et al. [1998]. The murine *GT1* gene is inducible with retinoic acid in the mouse embryonic carcinoma cell line P19 undergoing differentiation into neurons, with the highest expression in neurons and no expression in glial cells [Imai et al., 1995].

DISCUSSION

In this study, we excluded the role of CAG repeat expansions (in the range of those observed in neurodegenerative diseases caused by CAG repeat expansions) in 14 candidate genes, adding to the list of CAGrCG that were found not to be expanded in schizophrenia [Speight et al., 1997; Jain et al., 1996; Sasaki et al., 1996]. However, we identified a significant association between the *hGT1* gene and neuroleptic-responsive schizophrenia. Although the possibility that the CAG repeat in the *hGT1* gene may be functional as suggested for other genes [Chamberlain et al., 1994; Gerber et al., 1994], the most parsimonious interpretation of this finding is that allele –1 of the *hGT1* represents an anonymous marker in linkage disequilibrium with a nearby causative or modifier gene(s). The fact that allele –1, which accounts for most the difference between neuroleptic responder schizophrenic patients and controls, contains a CAA interruption in its sequence is consistent with this interpretation.

Because population-based case-control studies have several limitations, these results should be considered at best suggestive and interpreted with caution. First, although all patients and controls were Caucasians, it is possible that the association between neuroleptic-responsive schizophrenia and the *hGT1* gene was due to population stratification. The fact that the same pattern of results was observed in a smaller sample including only subjects of French Canadian ethnicity reduces, but does not necessarily eliminate, such bias. Second, the gender difference between R patients and controls could have resulted, at least partially, in the difference in allelic frequency between the two groups. However, there was no differences in allelic distribution between males and females in the control group and the same pattern of results was observed when controls and R patients were stratified according to gender (males adjusted $Z = -2.31$, $P = 0.02$; females: adjusted $Z = -2.10$, $P = 0.03$). Third, although we controlled for multiple testing, it is still possible that the association between neuroleptic responsive schizophrenia and *hGT1* gene might have arisen by chance. A replication of these results in an independent and larger sample, preferentially made of parent/child trios, will help to confirm the possible contribution of *hGT1*, a putatively neuron-specific gene [Imai et al., 1995], to the risk of schizophrenia and/or as a modifier of the phenotypic variability of schizophrenia with respect to neuroleptic response or long-term outcome.

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